JOURNAL OF VIROLOGY, Feb. 1985, p. 691–694
0022-538X/85/020691-04$02.00/0

Vol. 53, No. 2

NOTES

Cloning and Characterization of DNA Complementary to the Canine Distemper Virus mRNA Encoding Matrix, Phosphoprotein, and Nucleocapsid Protein

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Received 25 July 1984/Accepted 19 October 1984

Double-stranded cDNA synthesized from total polyadenylate-containing mRNA, extracted from monkey kidney cells infected with canine distemper virus (CDV), has been cloned into the *PstI* site of *Escherichia coli* plasmid pBR322. Clones containing canine distemper virus DNA were identified by hybridization to a canine distemper virus-specific, ^{32}P-labeled cDNA. Four specific clones containing different classes of sequences have been identified. The cloned plasmids contain inserts of 800 (clone 44-80), 960 (clone 74-16), 1,700 (clone 364), and 950 (clone 40-9) base pairs. The sizes of the mRNA species complementary to these inserts are 1,500, 1,850, 1,850 and 2,500 nucleotides, respectively, as determined by the Northern technique. Three of the cloned DNA fragments were further identified as the reverse transcripts of the mRNA coding for the matrix, phosphoprotein, and nucleocapsid protein of CDV.

Canine distemper virus (CDV) and measles virus (MV) are members of the morbillivirus subgroup of paramyxoviruses. Strong immunological cross-reactivity has been observed among some of the polypeptides (8, 9, 12, 16, 17). Both viruses can establish persistence in cell culture as well as in their natural hosts. We have undertaken a comparative study of these two morbilliviruses as an approach to the elucidation of possible molecular events that could lead to the alteration of these viruses from an acute infection to one producing a slow virus syndrome.

In the initial phase of this work, we had previously described measles virus polypeptides obtained upon in vitro translation of MV polyadenylate [poly(A)]-containing RNA (14); genetic engineering manipulation resulted in the cDNA cloning of three of the MV genes (2, 6, 14). In the present study, a comparison of polypeptide products (CDV and MV) synthesized in vitro is described, and the identification and characterization of CDV cDNA clones is demonstrated.

Poly(A)$^+$-containing RNA of CDV was used to direct polypeptide synthesis in a mRNA-dependent rabbit reticulocyte cell-free translation system. Shown in Fig. 1 are the in vitro products programmed by mRNA from measles- and CDV-infected cells. The assignments of the phosphoprotein (P), nucleocapsid protein (N), and matrix protein (M) of MV have been firmly established by monoclonal antibody and synthetic oligopeptide antisera in immunoprecipitation studies (2, 15). The assignments for the CDV polypeptide counterparts (designated N′, P′, and M′) have recently been reported by Barrett and Mahy (1). In addition, the assignments for the N and M proteins have been confirmed in our laboratory with the use of the well-documented cross-reactivity of CDV and MV structural proteins (12, 17). That is, antisera raised to synthetic oligopeptides constructed to portions of the amino acid sequence for the N and M proteins of MV precipitated the antigenically related CDV proteins (C. D. Richardson, A. Berkovich, S. Rozenblatt, and W. J. Bellini, J. Virol., in press; W. J. Bellini, G. Englund, C. D. Richardson, and S. Rozenblatt; manuscript in preparation). The CDV-P protein assignation was determined by immunoprecipitation with a monoclonal antibody to this polypeptide.

Some variation in the relative amounts of P, N, and M synthesized could be observed, reflecting the particular RNA preparation employed in the study. The following parameters were observed to affect the synthesis: (i) multiplicity of infection, (ii) length of time after infection before extraction of the RNA, and (iii) the type of susceptible cell line used. The largest variation observed was with the undefined polypeptide, designated X and X′ (Fig. 1, lanes A and C, B and E). The calculated molecular weight of CDV X′ polypeptide was 64,000 and that of the X protein of MV was 66,000. Recently, it has been shown that the nonglycosylated form of the measles virus hemagglutinin has a molecular weight of 65,000 (3), consistent with the in vitro-translated X product of MV. Although this may suggest that the X and X′ polypeptides are the nonglycosylated counterparts of the hemagglutinin proteins synthesized in vitro, no direct evidence to support this notion has been obtained.

A powerful approach for studying differential gene expression in cells infected with virus involves the development of nucleic acid hybridization probes that are gene specific. The population of double-stranded cDNA prepared from poly(A)$^+$-containing RNA, extracted from CDV-infected cells, was oligodeoxycytidylate-tailed and inserted into the appropriately tailed pBR322 at the *PstI* site. The circularized hybrid DNA was introduced into Ca^{2+}-sensitive *Escherichia coli* HB 101. Transformants (4,000 clones) resistant to tetracycline (15 μg/ml) were isolated, and clones containing CDV sequences were identified (384 clones) by a selective hybridization method adapted for screening clones that contain CDV-DNA sequences (6). Clones containing inserts larger

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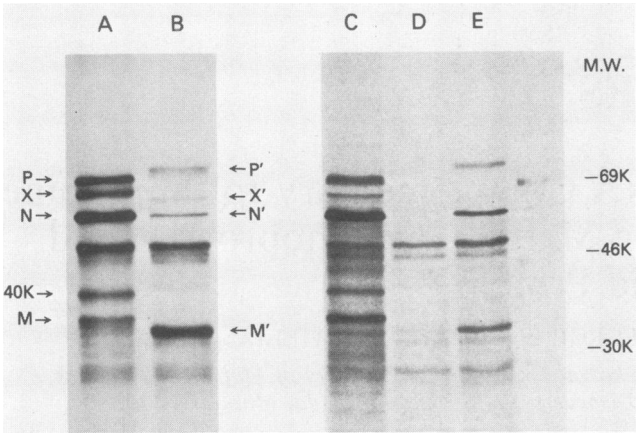

FIG. 1. In vitro synthesis of CDV polypeptides. The synthesis of [^{35}S]methionine-labeled polypeptides was programmed by mRNA from MV-infected cells (2 μg of RNA per 25-μl reaction volume) (lanes A and C), or mRNA from CDV-infected cells (lanes B and E), or mRNA from uninfected cells (lane D).

than 600 base pairs were 3′ end labeled with [^{32}P]cordycepin (11). These labeled fragments were tested for the presence of poly(A)$^+$ tails, using a binding assay based on the affinity to oligodeoxythymidylate. Inserts that bound to oligodeoxythymidylate-cellulose columns (50 μl) were then used to screen the CDV library. The 218 clones (56.8%) identified by this procedure could be divided into four groups based on the patterns of cross-hybridization. Clones containing the largest inserts from each of the four groups were employed for the remainder of this study. The insert sizes of each of the four clones chosen were determined by agarose gel electrophoresis after digestion of each cDNA clone with *Pst*I (Fig. 2). The representative clones for groups 1, 2, and 4 contained inserts of 800, 960, and 950 base pairs, respectively (Fig. 2, lanes B, C, and E). The representative of the group 3 clones contained an insert with an internal *Pst*I site and yielded two fragments of 1,100 and 600 base pairs (Fig. 2, lane D).

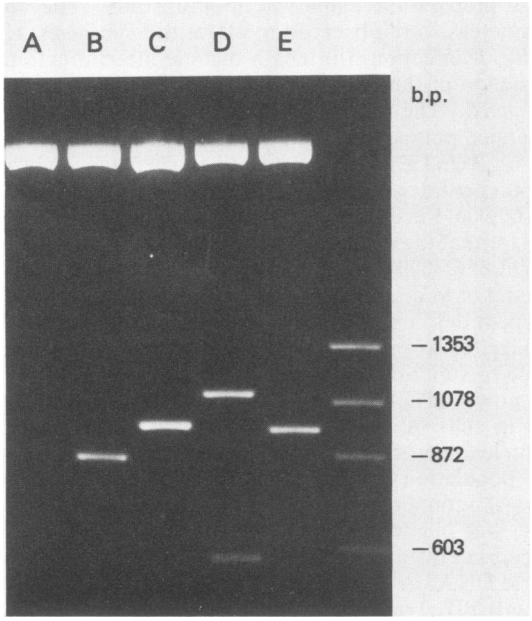

FIG. 2. Sizing of CDV DNA clones. Plasmid DNAs digested with *Pst*I were electrophoresed on a 1% agarose slab gel. Lanes: A, pBR322; B, clone 44-80; C, clone 74-16; D, clone 364; E, clone 40-9. ϕX174 DNA digested with *Hae*III was used for size markers.

The correlation of the CDV DNA clones with viral gene products was based upon DNA-mRNA hybrid-arrested and cell-free translation (13). DNA (10 μg) of the individual clones was separately digested with *Eco*RI and hybridized in solution with 2 μg of poly(A)$^+$-containing RNA from CDV-infected cells. Nucleic acid was ethanol precipitated and translated directly in an mRNA-dependent rabbit reticulocyte cell-free translation system (14). The synthesized radioactive polypeptides were fractionated on 10% sodium dodecyl sulfate polyacrylamide lab gels and exposed for autoradiography. The results (Fig. 3) demonstrated that clone 44-80 (group 1) (Fig. 3A, lane 3), clone 74 (group 2) (Fig. 3B, lane 1) clone 364 (group 3) (Fig. 3B, lane 2) almost completely abolished the synthesis of M, P, and N polypeptides of CDV, respectively. These results established that the clones representing groups 1, 2, and 3 contained cDNA sequences complementary for the mRNAs encoding the M, P, and N proteins of CDV. Therefore, we designated these clones CDV-M, CDV-P, and CDV-N. The cDNA clone representing group 4 did not detectably arrest the synthesis of any of the known CDV polypeptides synthesized in vitro. Thus, no designation for the coding function of this group could be made.

Determinations of the relative sizes of the mRNA reacting with each of the four clones was performed by Northern blot analysis. For each blot, mRNA (1 μg) from CDV-infected cells was fractionated under denaturing conditions, transferred, and fixed to nitrocellulose (4). Each of the identical blots was hybridized to one of the four probes. The N and P cDNA probes each hybridized to an mRNA with an approx-

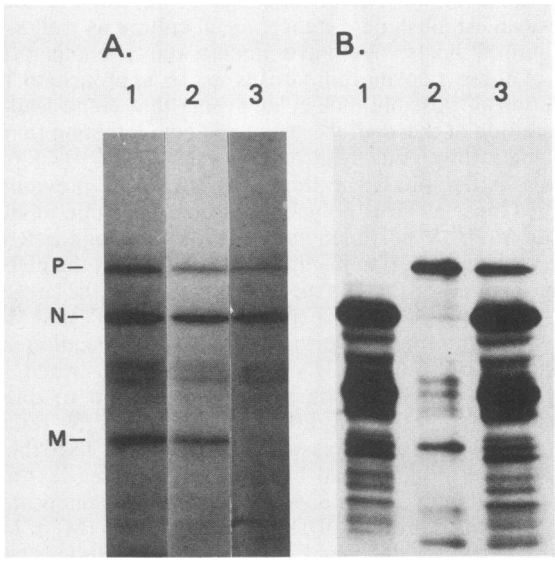

FIG. 3. Hybrid arrest of translation by CDV cDNA clones. Each of the representative cDNA plasmids (10 μg) or pBR322 DNA was digested with *Eco*RI and subsequently hybridized with mRNA (2 μg) from CDV-infected cells (12). After ethanol precipitation, the mRNA was used in in vitro translation studies. The polypeptides synthesized were analyzed on sodium dodecyl sulfate-polyacrylamide (10%) gels, fluorographically enhanced, dried, and exposed to X-ray film at −70°C. (A) Polypeptides synthesized after hybridization with no competing DNA (lane 1), pBR322 DNA (lane 2), CDV 44-80 (group 1) (lane 3). (B) Polypeptides immunoprecipitated (10) with rabbit anti-CDV serum after hybrid arrest and translation with CDV 74-16 (group 3) (lane 1), CDV 364 (group 3) (lane 2), and pBR322 (lane 3).

imate size of 1,850 bases (Fig. 4A, lanes N and P). Barrett and Mahy (1) have recently isolated a nucleocapsid cDNA clone of CDV which hybridizes to an mRNA of essentially the same size as that reported here. The matrix clone hybridized to an mRNA of 1,550 bases (Fig. 4A, lane M), whereas the representative of group 4 hybridized to an mRNA with an approximate size of 2,500 bases (Fig. 4A, lane *).

Under similar hybridization and filter washing conditions (50% formamide, 42°C, final wash in 0.1× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] at 65°C), no hybridization signals were observed with mRNA from uninfected cells or mRNA from MV-infected cells (data not shown). Figure 4B shows results obtained upon hybridization of three identical nitrocellulose blots containing 1 μg of mRNA from MV-infected cells hybridized with the following MV DNA probes: nucleocapsid (Fig. 4B, lane "N"), phosphoprotein (Fig. 4B, lane "P"), and matrix (Fig. 4B, lane M) (1, 5, 13). It should be noted that the sizes of the mRNAs specifically encoding the M, P, and N gene products are essentially identical for CDV and MV, despite differences in the apparent molecular weight of the M and P proteins of these viruses (Fig. 1). The minor difference in the migration of CDV M (34 kilodaltons [kd]) and measles M (36 kd) could easily be explained by the inability to distinguish differences in the mRNA of only 60 nucleotides. However, the more pronounced differences in the migration of the P proteins, of CDV (78 kd) and MV (70 kd), cannot be explained on this basis. It is likely that the apparent molecular weights of these proteins do not accurately reflect the true molecular weights. Giorgi et al. (5) have cloned and sequenced the phosphoprotein of Sendai virus. The calculated molecular weight from the predicted amino acid sequence (62,011) was significantly different from the apparent molecular weight (79,000) determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. We have recently completed the sequence analysis of the measles P gene. Again, the predicted molecular weight of 53,964 is far less than the 70,000 figure determined by acrylamide gel electrophoresis (W. J. Bellini, G. Englund, S. Rozenblatt, H. Arnheiter, and C. D. Richardson, J. Virol., in press).

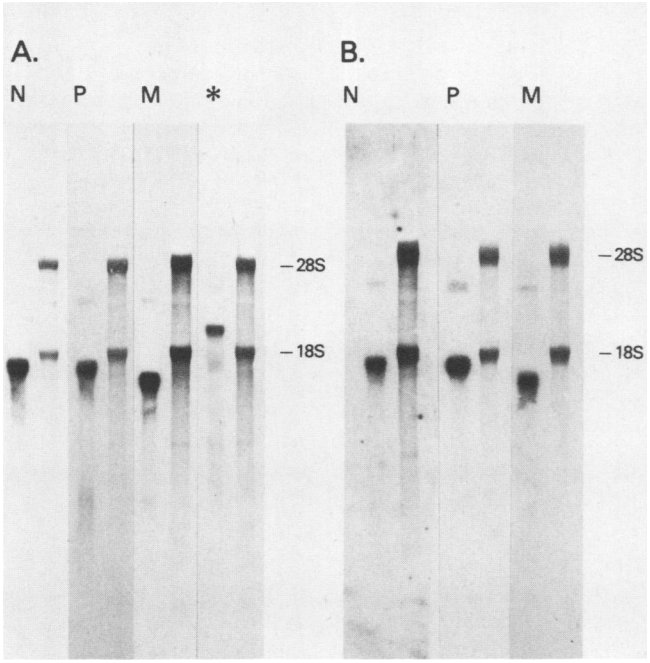

FIG. 4. Sizing of CDV mRNAs. Poly(A)$^+$ RNA (2 μg) from CDV-infected cells (A) and MV-infected cells (B) were fractionated under denaturing conditions in a 1% agarose slab gel (4). The RNA was transferred to nitrocellulose paper and hybridized in vitro with [^{32}P]cordycepin 3′-end-labeled DNA. (A) CDV cDNA clones: clone 364 (group 3, lane N); clone 74-16 (group 2, lane P); clone 44-80 (group 1, lane M); clone 40-9 (group 4, lane *). (B) MV cDNA clones: clone 15 (lane N); clone p11 (lane P); clone M-5 (lane M). ^{14}C-labeled 185 and 185 rRNAs were used as markers.

TABLE 1. Nucleotide sequences at the very 3′ end of CDV and MV deduced mRNA

mRNA size (bases)	Protein coded for[a]	CDV sequence	MV sequence
1,850	N	UUAU(A)$_n$	UUAU(A)$_n$
1,850	P	UUAU(A)$_n$	UUAU(A)$_n$
1,500	M	AAUC(A)$_n$	AAAC(A)$_n$
2,500	*	AAAG(A)$_n$	—[b]

[a] Protein abbreviations are defined in the text.
[b] —, Sequence not available.

In the process of selecting the representative cDNA clones for each of the four groups, we chose those clones with the largest CDV inserts. We have sequenced the very 3′ ends (message sense) of each of these representative groups and have compared their sequence with that of MV cDNA clones representing the 3′ ends of the N, P, and M genes. Several nucleotides adjacent to the poly(A)$^+$ tract of the mRNAs of the different genes in CDV and MV are summarized in Table 1. These sequences are based not only on the four clones representing the four CDV groups identified, but also on additional clones of each group encompassing the same region. The same is true for the MV sequences reported in Table 1. Both the 3′-end sequences of the N and P clones for CDV and MV contain the identical tetranucleotide 5′-UUAU-3′ directly adjacent to the poly(A)$^+$ tract. Interestingly, neither of the M genes shares this tetranucleotide, nor does the 3′-end sequence of the CDV group 4 clones. These findings are unlike those for the related virus, Sendai, in which all of the known sequences at the very 3′ end of the mRNAs are identical (7). One possible role for the variability observed is the governing of mRNA synthesis for differential gene expression. These differences in the attenuation-polyadenylation signals may have profound effects on the efficiency of transcription of the downstream messages.

S.R. and W.J.B. thank R. A. Lazzarini for his support and encouragement.

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